Sedative and Analgesic Effects of Actinidiae Stem

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ABSTRACT

In this study, we extracted *Actinidia chinensis* Planch. var. *setosa* Li (AC) stem with 95 % ethanol. The ethanol extract was then partitioned with varying organic solvents to obtain chloroform, ethyl acetate, *n*-butanol and water fractions. Behavioral methods were then employed to investigate the sedative and analgesic effects and the mechanism of action of each fraction.

The ethanol extract of AC and the four solvent fractions possessed low toxicity. The ethanol extract prolonged the sleeping time induced by hexobarbital in mice and reduced the motor activity of rats. The water fraction had the strongest sedative effect. None of the ethanol extracts displayed muscle relaxant activity. Both the ethanol extract and the water fraction increased the hot plate-induced pain response time and acetic acid-induced writhing response. The water fraction also enhanced the analgesic effect produced by baclofen and potentiated the hypomotility produced by 5-hydroxytryptophan (5-HTP) and baclofen.

These results show that AC possesses sedative and analgesic activities. Of the four solvent fractions, the water fraction exhibited the strongest sedative and analgesic activities. We speculate that its sedative effects might be mediated by increases in serotonergic and GABA-Bergic activities whereas its analgesic effects could be due to increases in GABA-Bergic activities

Key words: Actinidiae stem; Sedation; Analgesia.

INTRODUCTION

The roots of *Actinidia chinensis* Planch. var. *setosa* Li (AC) have been used in Taiwan folk medicine for treating hepatitis and insomnia.[1] Insomnia and pain are two complaints which lead patients to seek medical attention. Unfortunately, all the drugs with hypnotic and analgesic effects possess side effects. The search for less toxic drug to treat insomnia and pain is an ongoing goal of our pharmacomedical staff. The sedative and analgesic ef-

fects of AC stems have never been reported. Therefore, in the present study, we extracted AC stems with 95 % ethanol and then partitioned the ethanol extract with different organic solvents to obtain chloroform, ethyl acetate, *n*-butanol and water fractions. Behavioral pharmacological methods were then employed to investigate the sedative and analgesic effects and the mechanisms of action of the fractions.

MATERIALS AND METHODS

Preparation of Actinidiae Stems

Actinidia chinensis Planch. var. *setosa* (Actinidiaceae) was collected from Ts'ui-Fung mountain in Nan-T'ou County and was authenticated by Nien-Yung Chiu, Technician, Institute of Chinese Pharmaceutical Sciences, China Medical College. The stems were chopped, dried, extracted and partitioned to obtain an ethanol extract as well as chloroform, ethyl acetate, *n*-butanol and water fractions as illustrated in Fig. 1.

Animals

Male ICR mice, weighing between 20-25 g, were used to determine acute toxicity, and

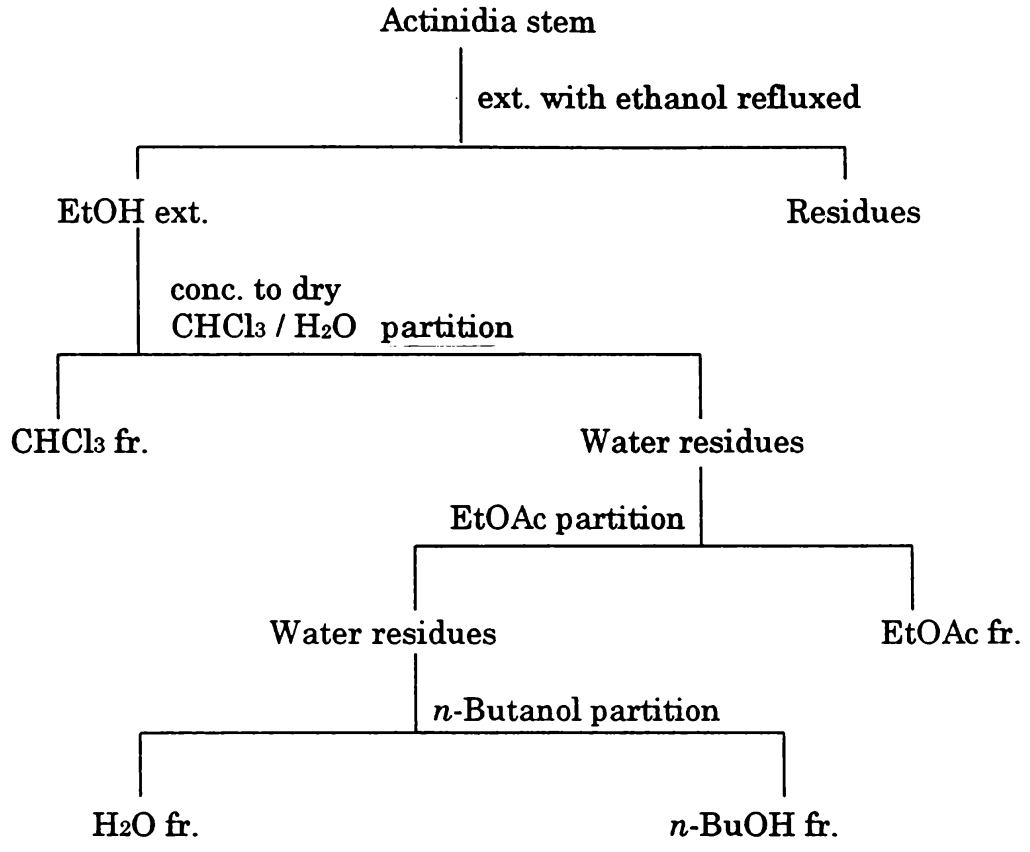

Fig. 1. Separation of Actinidia stems EtOH ext.: ethanol extract of AC
$CHCl_3$ fr.: chloroform fraction of EtOH ext. EtOAc fr: ethylacetate fraction of EtOH ext.
n-BuOH fr: *n*-butanol fraction of EtOH ext. H_2O fr.: water fraction of EtOH ext.

analgesic, muscle relaxant, and sedative effects of the fractions. Male Sprague-Dawley rats, weighing between 200-250 g, were used to examine the effects of the fractions on motor activity. The animals were maintained at a controlled temperature of 22 ± 1 °C, humidity of 55 ± 5 %, and housed in a metal-wire cage for a minimum of 7 days prior to each experiment. Solid diets (Fu Shou Co., Taipei) and tap water were provided.

Acute Toxicity

A sufficient number of mice were used to measure the oral and intraperitoneal toxicity of the ethanol extract of AC and its fractions in this study. All deaths were recorded during a 72-h period. Ten mice per dose were used to obtain estimates of the LD50 using the method of Litchfield and Wilcoxon.[2]

Effects on Hexobarbital-induced Sleep

The AC ethanol extract and solvent fractions were administered orally to mice at doses of 0.1, 0.5 and 2.5 g/kg. Sixty minutes later, hexobarbital (100 mg/kg, i.p.) was administered to induce sleep. The hypnotic effect index was measured by the interval between loss and recovery of the righting reflex.[3] In the control group, administration of hexobarbital was substituted with normal saline.

Measurement of Motor Activity

The motor activity of individual rats was measured with a MK-ANIMEX activity meter (Muromachi Kikai Co., Japan). The sensitivity and tuning of the instrument were adjusted to 35 μA to enable all types of motor behavior, including locomotion, rearing, grooming, sniffing and licking to be jointly recorded. Activity was recorded for 2 hours, beginning 5 minutes after inserting the animal into the test cage. Each rat was used only once and a total of six rats were used for each treatment.[4] The ethanol extract of AC and the solvent fractions (0.1, 0.5 g/kg) were administered orally. Sixty minutes later, motor activity was recorded. α-Methyl-*p*-tyrosine (α-MT), apomorphine (APO), 5-hydroxytryptophan (5-HTP), baclofen (BAC) and muscimol (MUS) were used to test their effects on the subsequent changes of motor activity produced by the water fraction. APO was dissolved in an ascorbic acid solution, 5-HTP was suspended in 5 % CMC whereas α-MT, BAC and MUS were dissolved in 0.9 % saline. APO, 5-HTP, BAC and MUS were prepared immediately before use. The following compounds were given prior to administration of the water fraction (0.5 g/kg, p.o.) as follows: α-MT (50 mg/kg, i.p.) 2 hours prior;[5] APO (0.3 mg/kg, i.p.) 10 minutes prior;[6] 5-HTP (50 mg/kg, i.p.) 30 min prior;[7] BAC (0.5 mg/kg, i.p.) 10 min prior;[8] MUS (0.3 mg/kg, i.p.) 10 min prior.[9] Each rat was placed in the test cage 60 min after the administration of AC. All of the test drugs were purchased from Sigma

Chemical Co.

Muscle Relaxant Effects

The ethanol extract of AC and the water fraction were administered orally to the mice at doses of 0.1, 0.5 g/kg The time period required to hook the back-paws on the iron-wire was used as an index of muscle relaxation and was measured at 30 min intervals for a period of 120 mins.[10] Normal saline was administered in the control group.

Analgesic Effects

Hot plate method

The ethanol extract of AC or the solvent fractions (0.1, 0.5 g/kg, p.o.) were first administered. Thirty min later, mice were placed on a hot plate (55 ± 0.5 °C). The latency of heat induced pain response was measured. Jumping, licking, or withdrawal of back-paws were used as the indices of pain and were measured at 30 min intervals. Normal saline was administered in the control group.

Acetic acid method

The ethanol extract of AC or the solvent fractions (0.1, 0.5 g/kg, p.o.) were administered. One hour later, 1 % (v/w) acetic acid (0.1 mL/10 g) was given intraperitoneally to produce pain. The frequency of writhing was used as an index of pain and was measured at 5 min intervals. Normal saline was administered in the control group.

BAC (0.5 mg/kg, i.p.) and MUS (0.5 mg/kg, i.p.) were used to test their respective effects on the changes of pain response produced by the water fraction in both the hot plate and the acetic acid methods.

Statistical Analysis

Results were statistically analyzed by using ANOVA followed by Duncan's multiple range test to compare differences. A P value below 0.05 was defined as statistically significant.

RESULTS

Acute Toxicity Studies

Table 1 shows the oral and intraperitoneal LD_{50} and 95 % confidence limits of the ethanol extract of AC and its solvent fractions. Mice recieved oral doses of the ethanol extract of AC or the solvent fractions at 10 g/kg and were then observed for 72 h. Hypomotility, dyspnea and finally anoxic convulsions and death were observed after intraperitoneal administration, with death usually occurring within 2 h after AC administration.

Effects on Hexobarbital-induced Sleep

As shown in Table 2, the ethanol extract as well as the chloroform and water fractions prolonged the hexobarbital-induced sleeping time in mice in a dose-dependent fashion.

Effects on Motor Activity

The ethanol extract of AC and the solvent fractions decreased the motor activity of the

Table 1. LD_{50} and 95 % Confidence Limits of AC Extracts in Mice

Drugs	Route	LD_{50} (g/kg)	(95 % confidence limits)
EtOH ext.	p.o.	>10	
	i.p.	2.20	(1.82 - 2.67 g/kg)
$CHCl_3$ fr.	p.o.	>10	
	i.p.	2.45	(1.70 - 3.54 g/kg)
EtOAc fr.	p.o.	>10	
	i.p.	1.35	(1.04 - 1.67 g/kg)
n-BuOH fr.	p.o.	>10	
	i.p.	1.50	(1.07 - 2.12 g/kg)
H_2O fr.	p.o.	>10	
	i.p.	3.56	(3.25 - 3.90 g/kg)

Table 2. Effect of AC Extracts on the Hexobarbital-induced Hypnosis in Mice

Drugs	Dose (g/kg, p.o.)	Onset (min.)	Sleeping time (min)
Control		3.63 ± 0.26	24.48 ± 1.30
EtOH ext.	0.1	3.37 ± 0.19	25.49 ± 2.02
	0.5	3.63 ± 0.24	39.28 ± 2.50**
$CHCl_3$ fr.	0.1	3.62 ± 0.09	27.10 ± 1.32
	0.5	3.63 ± 0.24	35.63 ± 1.84**
EtOAc fr.	0.1	3.62 ± 0.09	29.80 ± 2.18
	0.5	3.68 ± 0.15	28.63 ± 2.58
n-BuOH fr.	0.1	3.93 ± 0.22	25.46 ± 1.12
	0.5	3.26 ± 0.20	25.47 ± 1.92
H_2O fr.	0.1	3.17 ± 0.10	30.78 ± 1.71
	0.5	3.47 ± 0.19	36.60 ± 2.03**

Data represented as mean ± SEM for 6 rats. Onset: righting reflex disappear.
Sleeping time: time from onset to recovery of the disappearance of righting reflex.
**$p < 0.01$, compared with the control group (ANOVA for repeated measures followed by Duncan's multiple range test).

Table 3. Effect of AC Extracts on the Motor Activity in Rats

Drugs	Dose (g/kg)	30 min.	60 min.	90min.
Control		570.50 ± 27.79	118.00 ± 10.31	87.17 ± 3.66
EtOH ext.	0.1	529.00 ± 15.58	120.33 ± 7.80	83.33 ± 4.88
	0.5	353.33 ± 15.94**	103.33 ± 2.28	77.67 ± 4.39
$CHCl_3$ fr.	0.1	583.00 ± 16.21	117.33 ± 5.73	92.17 ± 2.92
	0.5	569.17 ± 11.09	118.67 ± 7.91	88.00 ± 2.24
EtOAc fr.	0.1	559.50 ± 20.96	121.50 ± 7.88	90.17 ± 2.90
	0.5	582.33 ± 16.00	122.00 ± 6.86	89.50 ± 3.21
n-BuOH fr.	0.1	592.33 ± 12.74	111.00 ± 3.13	88.00 ± 2.84
	0.5	594.67 ± 5.311	112.67 ± 2.72	86.33 ± 3.24
H_2O fr.	0.1	532.50 ± 17.67	115.33 ± 7.99	86.00 ± 3.30
	0.5	381.33 ± 5.33**	97.50 ± 1.28	71.33 ± 1.26

Data represented as mean ± SEM for 6 rats.

**p < 0.01, compared with control group (ANOVA for repeated measures followed by Duncan's multiple range test)

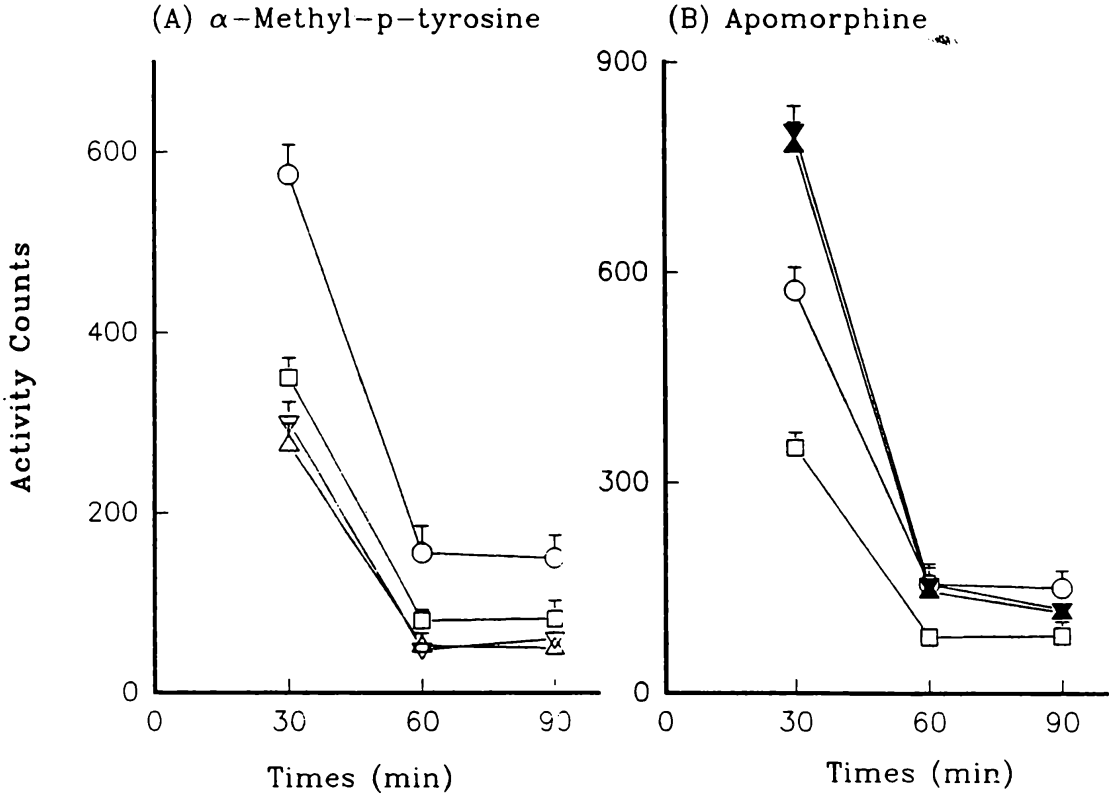

Fig. 2. Effect of H_2O fraction on the changes in motor activity produced by (A) α-methyl-*p*-tyrosine and (B) apomorphine in rats. □, H_2O fraction (0.5 g/kg, p.o., 1 hr in advance). ∇, α-methyl-*p*-tyrosine (50 mg/kg, i.p., 2 hr in advance). Δ, α-methyl-*p*-tyrosine plus H_2O fraction. ▼, apomorphine (0.3 mg/kg, i.p., 10 min in advance). ▲, apomorphine plus H_2O fraction. O, control.

mice after about 30 min. The water fraction had the strongest depressive effect and exhibited significant dose-response pattern (Table 3).

As shown in Fig. 2, rats treated with α-MT alone exhibited behavioral depression. The water fraction had no significant effect on the hypomotility produced by α-MT. APO produced a significant increase in motor activity over control values at all time intervals. The water fraction also did not influence the APO-induced hypermotility.

Rats treated with 5-HTP alone exhibited behavioral depression. The hypomotility produced by 5-HTP was significantly augmented by the water fraction (Fig. 3).

As shown in Fig. 4, rats treated with BAC or MUS appeared to be behaviorally depressed. The hypomotility produced by BAC was significantly augmented by the water fraction. However, the water fraction had no influence on the hypomotility produced by MUS.

Table 4. Analgesic Effect of AC Extracts in Mice (Hot Plate Method)

Treatment		30 min.	60 min	90 min.
Control		-1.92 ± 1.64	1.81 ± 0.57	2.04 ± 3.42
EtOH ext.	0.1 g/kg	0.53 ± 1.71	3.37 ± 0.42	1.87 ± 2.03
	0.5 g/kg	2.11 ± 1.10	10.43 ± 1.73**	6.68 ± 1.74
$CHCl_3$ fr.	0.1 g/kg	-0.79 ± 2.73	3.53 ± 1.92	0.32 ± 2.47
	0.5 g/kg	2.19 ± 1.93	3.19 ± 0.18	4.20 ± 1.60
EtOAc fr.	0.1 g/kg	-0.36 ± 1.39	3.14 ± 0.93	2.47 ± 1.14
	0.5 g/kg	0.51 ± 0.92	5.52 ± 1.46	2.13 ± 0.63
n-BuOH fr.	0.1 g/kg	2.54 ± 5.01	3.79 ± 1.42	1.04 ± 2.59
	0.5 g/kg	4.03 ± 3.32	4.13 ± 2.30	1.63 ± 2.51
H_2O fr.	0.1 g/kg	1.23 ± 2.85	4.21 ± 2.13	3.84 ± 2.31
	0.5 g/kg	0.47 ± 2.52	12.42 ± 1.74**	6.32 ± 2.44
BAC	0.5 mg/kg	3.52 ± 1.21	8.79 ± 2.43	5.14 ± 2.53
BAC + H_2O fr.	0.1 g/kg	0.93 ± 0.91	6.03 ± 2.42	6.06 ± 1.12
	0.5 g/kg	1.13 ± 0.14	19.19 ± 2.04**	11.34 ± 0.83*
MUS	0.5 mg/kg	6.53 ± 1.52	8.52 ± 0.74	6.53 ± 1.82
MUS + H_2O fr.	0.1 g/kg	1.52 ± 2.73	10.61 ± 2.23	3.94 ± 1.14
	0.5 g/kg	2.84 ± 3.21	12.34 ± 1.41	6.32 ± 0.83

Data represented as mean ± SEM for 6 rats.

$$\text{Mean: } \frac{\text{Time of Post administration - Time of Pre administration}}{\text{Time of Pre administration}} \text{ 100 \%}$$

*$p < 0.05$, **$p < 0.01$, compared with control group (ANOVA for repeated measures followed by Duncan's multiple range test)

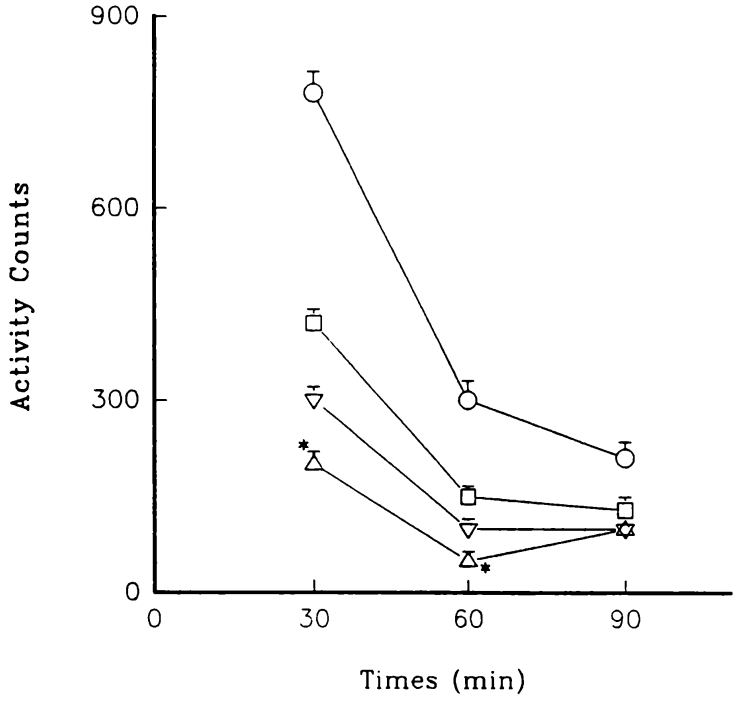

Fig. 3. Effect of the H_2O extract on the changes in motor activity produced by 5-hydroxytrypotophan in rats. □, H_2O fraction (0.5g/kg, p.o., 1 hr in advance). ∇, 5-hydroxytrypotophan (50 mg/kg, i.p., 30 min in advance). Δ,, 5-hydroxytrypotophan plus H_2O fraction. O, control. *$p < 0.05$ compared with 5-hydroxytryptophan (∇) group (ANOVA for repeated measures followed by Duncan's multiple range test; n=6).

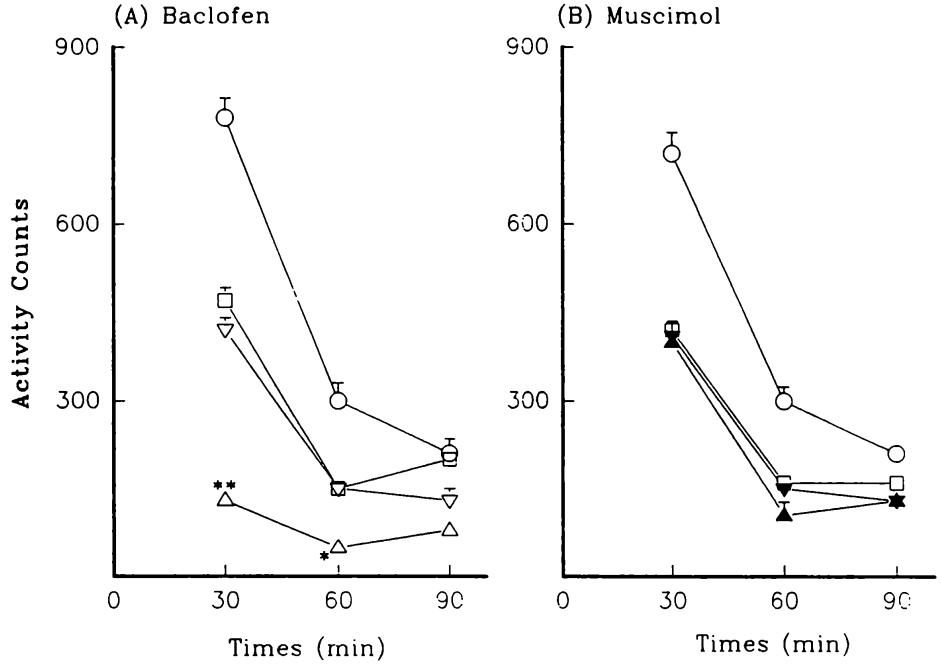

Fig. 4. Effect of the H_2O fraction on the changes in motor activity produced by (A) baclofen and (B) muscimol in rats. □, H_2O fraction (0.5 g/kg, p.o., 1 hr in advance). ∇, baclofen (0.5 mg/kg, i.p., 10 min in advance). Δ, baclofen plus H_2O fraction. ▼, muscimol (0.3 mg/kg, i.p., 10 min in advance). ▲, muscimol plus H_2O fraction. O, control. *$p < 0.05$, **$p < 0.01$ compared with baclofen (∇) group (ANOVA for repeated measures followed by Duncan's multiple range test; n=6).

Analgesic Effects

Hot plate method

As shown in Table 4, both the pain thresholds of both the ethanol extract and water fraction groups were elevated at 60 min ($p < 0.01$).

Treatment of rats with BAC or MUS, elevated the pain thresholds from 60 min to 90 min. The analgesic effect of BAC was augmented by the water fraction. The water fraction did not affect the analgesic effect of MUS.

Acetic acid method

As shown in Table 5, the ethanol extract and the water fraction inhibited the writhing response induced by 1 % acetic acid. BAC (0.5 mg/kg) inhibited the writhing response induced by 1 % acetic acid. The water fraction augmented the analgesic effect produced by

Table 5. Analgesic Effect of AC Extracts on the Acetic Acid-induced Writhing Response in Mice

Drugs	Dose (g/kg, p.o.)	Total no. of writhing for 10 min.
Control		41.83 ± 1.45
EtOH ex.	0.1	39.20 ± 2.29
	0.5	31.60 ± 1.29**
$CHCl_3$ fr.	0.1	35.20 ± 6.47
	0.5	39.80 ± 2.22
EtOAc fr.	0.1	42.40 ± 2.99
	0.5	43.00 ± 4.66
n-BuOH fr.	0.1	43.60 ± 2.14
	0.5	43.20 ± 3.51
H_2O fr.	0.1	38.60 ± 2.69
	0.5	32.33 ± 2.68**
BAC	0.5 mg/kg, i.p.	35.10 ± 1.42
BAC + H_2O fr.	0.1	35.24 ± 0.92
	0.5	25.28 ± 2.53##
MUS	0.5 mg/kg, i.p.	30.40 ± 1.24
MUS + H_2O fr.	0.1	32.21 ± 0.92
	0.5	29.39 ± 2.15

Data represented as mean ± SEM for 6 rats.

**$p < 0.01$, compared with control group (ANOVA for repeated measures followed by Duncan's multiple range test)

##$p < 0.01$, compared with BAC group (ANOVA for repeated measures followed by Duncan's multiple range test).

Table 6. Effect of the Ethanol Extract of AC on Traction Performance in Mice

Drugs	Dose (g/kg, p.o.)	No. of mice treated	No. of mice which fell within 5 seconds after drug administration			
			30	60	90	120 (min.)
Control		6	0	0	0	0
EtOH ext.	0.1	6	1	1	0	0
	0.5	6	1	1	1	0
$CHCl_3$ fr.	0.1	6	1	1	0	0
	0.5	6	1	1	1	0
EtOAc fr.	0.1	6	1	1	0	0
	0.5	6	1	1	1	0
n-BuOH fr.	0.1	6	1	1	0	0
	0.5	6	1	1	1	0
H_2O fr.	0.1	6	1	1	0	0
	0.5	6	1	1	0	0

BAC. MUS (0.5 mg /kg) inhibited the writhing response induced by 1 % acetic acid, whereas, the water fraction had no effect on the MUS-induced analgesic effect.

Muscle Relaxant Effect

As shown in Table 6, the ethanol extract of AC and the water fraction had no significant muscle relaxant effect.

DISCUSSION

AC is commonly found in the mountains of Taiwan. The AC fruit is well known to the local population. The AC stem has been used by the traditional Chinese physicians to cure insomnia. In this study, we found that the ethanol extract of AC as well as its water fraction had low toxicity after oral administration, prolonged the hexobarbital-induced sleeping time, hot plate-induced pain response time and acetic acid-induced writhing response in mice, and decreased motor activity in rats. However, the ethanol extract of AC and the four solvent fractions did not induce muscle relaxation. These results imply that the ethanol extract of AC and the water fraction possessed sedative and analgesic activities. The sedative effects of the ethanol extract and the water fraction were not due to muscle relaxant effects. Furthermore, the water fraction had the strongest sedative effect. Therefore, the relationship between the sedative mechanism of the water fraction and its monoaminergic activity were investigated.

The relation between the sedative effect of the water fraction and its monoaminergic

system activity was investigated by using α-MT (an inhibitor of tyrosine hydroxylase), APO (a DA receptor agonist) and 5-HTP (a precursor of serotonin). α-MT pretreatment depletes the pool of newly-synthesized catecholamines[5] and reduces motor activity,[11-13] whereas APO produces hypermotility.[6] The water fraction had no effect on the α-MT- and APO-induced motor activity changes. 5-HTP can increase the central 5-HT levels with a subsequent decrease in motor activity.[7] The water fraction augmented the hypomotility produced by 5-HTP. This result implied that the water fraction acted to increase the serotonergic activity.

GABA receptors are divided into the GABA-A and GABA-B receptor subtypes.[14] In order to investigate the relationship of the sedative and analgesic effects of the water fraction and GABAergic system activity, the GABA-A receptor agonist (MUS) and GABA-B receptor agonist (BAC) were used. Both MUS and BAC decrease motor activity[15,16] and produce an analgesic effect.[17] The water fraction augmented the hypomotility and the analgesic effect produced by BAC, but had no effect on the hypomotility and analgesic effect produced by MUS. GABAergic ligands produce analgesia under many different conditions. These effects are probably mediated by GABA-B receptors or through other transmitter systems.[12] Blockade of catecholamines with systemic injection of α-methyl-*p*-tyrosine enhances baclofen analgesia.[18] The water fraction had no effect on the α-MT-induced motor activity changes. These results imply that the hypomotility and analgesic effects of the water fraction may be mediated via GABA-B receptors.

In conclusion, the ethanol extract of AC and the water fraction had sedative and analgesic effects. The mechanism of the sedative effect induced by the water fraction may be related to increase in serotonergic and GABA-Bergic activities. The analgesic mechanism of the water fraction may be related to increases in GABA-Bergic activities. Therefore, the active constituents of AC will be separated from the water fraction by column chromatography in the future. A better understanding of the exact sedative and analgesic mechanisms in the peripheral or central nervous systems is a goal of future research.

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